

Amendments to the Specification:

Please replace the paragraph beginning at page 14, line 28 with the following amended paragraph:

A "purified" Hsp60 stress protein of the present invention is a heat shock protein of the Hsp60 family from *Streptococcus pneumoniae* or *Streptococcus pyogenes* that has been purified from its producing cell. For example, the Streptococcal Hsp60 polypeptides of the present invention can be purified by a variety of standard methods with or without a detergent purification step. For example, Streptococcal Hsp60 can be isolated by, among other methods, culturing suitable host and vector systems to produce recombinant Hsp60 (discussed further herein). Then, supernatants from such cell lines, or Hsp60 inclusions, or whole cells where the Hsp60 is not excreted into the supernatant, can be treated by a variety of purification procedures. For example, the Streptococcal Hsp60-containing composition can be applied to a suitable purification matrix such as an anti-Hsp60 antibody bound to a suitable support. Alternatively, anion or cation exchange resins, gel filtration or affinity, hydrophobic or reverse phase chromatography may be employed in order to purify the protein. The Hsp60 polypeptide can also be concentrated using commercially available protein concentration filters, such as an Amicon or Millipore Pellicon PELLICON ultrafiltration unit, or by vacuum dialysis. In another alternative the supernatant can first be concentrated using one of the above mentioned protein concentration filters, followed by application of the concentrate to a suitable purification matrix such as those described above.

Please replace the paragraph beginning at page 17, line 14 with the following amended paragraph:

Bacterial expression vectors preferably comprise a promoter, which functions in the host cell, one or more selectable phenotypic markers, and a bacterial origin of replication. Representative promoters include the β -lactamase (penicillinase) and lactose promoter system (see Chang *et al.*, *Nature* 275:615, 1978), the T7 RNA polymerase promoter (*Studier et al.*, *Meth. Enzymol.* 185:60-89, 1990) the lambda promoter (*Elvin et*

al., *Gene* 87123-126, 1990), the *trp* promoter (Nichols and Yanofsky, *Meth. In Enzymology* 101:155, 1983) and the *tac* promoter (Russell et al., *Gene* 20:231, 1982). Representative selectable markers include various antibiotic resistance markers such as the kanamycin or ampicillin resistance genes. Many plasmids suitable for transforming host cells are well known in the art, including among others, pBR322 (see Bolivar et al., *Gene* 2:95, 1977), the pUC plasmids pUC18, pUC19, pUC118, pUC119 (see Messing, *Meth. in Enzymology* 101:20-77, 1983; Vieria and Messing, *Gene* 19:259-268, 1982), and pNH8A, pNH16a, pNH18a, and Bluescript ~~BLUESCRIPT~~ M13 (Stratagene, La Jolla, Calif.).

Please replace the paragraph beginning at page 25, line 4 with the following amended paragraph:

Cells that are obtained through the use of *in vitro* immunization or from an immunized animal as described above may be immortalized by transfection with a virus such as the Epstein-Barr Virus (EBV). (See Glasky and Reading, *Hybridoma* 8(4):377-389, 1989.) Alternatively, within a preferred embodiment, the harvested spleen and/or lymph node cell suspensions are fused with a suitable myeloma cell in order to create a "hybridoma" which secretes monoclonal antibodies. Suitable myeloma lines are preferably defective in the construction or expression of antibodies, and are additionally syngeneic with the cells from the immunized animal. Many such myeloma cell lines are well known in the art and may be obtained from sources such as the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209 (see *Catalogue of Cell Lines & Hybridomas*, 6th ed., ATCC, 1988). Representative myeloma lines include: for humans UC 729-6 (ATCC No. CRL 8061), MC/CAR-Z2 (ATCC No. CRL 8147), and SKO-007 (ATCC No. CRL 8033); for mice, SP2/0-Ag14 (ATCC No. CRL 1581), and P3X63Ag8 (ATCC No. TIB 9); and for rats, Y3-Ag1.2.3 (ATCC No. CRL 1631), and YB2/0 (ATCC No. CRL 1662). Particularly preferred fusion lines include NS-1 (ATCC No. TIB 18) and P3X63 – Ag 8.653 (ATCC No. CRL 1580), which may be utilized for fusions with either mouse, rat, or human cell lines. Fusion between the myeloma cell line and the cells from the immunized animal can be

Applicant : Lee Mizzen *et al.*
Serial No. : 09/001,737
Filed : December 31, 1977
Page : 4

Attorney's Docket No.: 12071-014001

accomplished by a variety of methods, including the use of polyethylene glycol (PEG) (*see Antibodies: A Laboratory Manual*, Harlow and Lane, *supra*) or electrofusion. (*See* Zimmerman and Vienken, *J. Membrane Biol.* 67:165-182, 1982.)

Please replace the paragraph beginning at page 37, line 23 with the following amended paragraph:

Genomic DNA from *Streptococcus pneumoniae* (ATCC6314) and *Streptococcus pyogenes* (ATCC12344), prepared by a routine method, ~~was obtained~~ was obtained from Dr. Lee Weber, University of Nevada at Reno.

Please replace the paragraph beginning at page 43, line 16 with the following amended paragraph:

Lysate was cleared by centrifugation at 10,000 x g for 15 min (at 4°C). The supernatant solution was mixed overnight at room temperature with approximately 100ml of slurry containing 50ml of ~~Ni-Sephadex~~ Ni-SEPHAROSE (Chelating Sepharose, Pharmacia) equilibrated in buffer A. The resin was then washed on filter paper with approximately 200 ml buffer A, resuspended in small volume of the same buffer and gravity-packed into glass chromatography column (Pharmacia).

Please replace the paragraph beginning at page 43, line 22 with the following amended paragraph:

The column was washed with 20 ml of buffer A with 1% ~~Triton~~ TRITON X-100. The column was further washed with a 6 - 0 M guanidinium hydrochloride / 0 - 1 M NaCl gradient in 50mM Tris-HCl pH 7.5, 0.5 mM beta-mercaptoethanol (200ml), then with 200 ml of 50 mM Tris-HCl pH 7.5, 1 M NaCl, 0.5 mM beta-mercaptoethanol, and finally with 200 ml of 50 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl pH 7.5, 1.025 M NaCl, 0.5 mM beta-mercaptoethanol. Then column was developed with a 200ml-gradient from 5% to 100% of buffer composed of 1 M imidazole, 0.5 M NaCl, 50mM Tris-HCl pH 7.5, 0.5 mM beta-mercaptoethanol in 1M NaCl, 50mM Tris-HCl pH 7.5, 0.5

mM beta-mercaptoethanol. Fractions of 9ml were collected. The flow rate was 4-5 ml/min, and chromatography was monitored by absorbance at 280 nm.

Please replace the paragraph beginning at page 45, line 21 with the following amended paragraph:

Samples containing 0.1 μ g, 0.5 μ g or 1 μ g of recombinant protein were fractionated on 10% SDS-PAGE, and proteins were electroblotted onto nitrocellulose. Blots for analysis with antibodies SPA-804, SPA-807, SPA-870, and anti-BCG Hsp60 were blocked with 5% skim milk in PBS containing 0.05% ~~Tween~~ TWEEN 20 overnight at room temperature. Blots were then incubated for one hour in the same buffer containing primary antibody (at a 1:1000 dilution except for anti-BCG Hsp60 antibody which was used at 1:500 dilution). Blots were washed 3 times (10 min each) with PBS with 0.05% TWEEN 20 and incubated for an additional hour in PBS with 5% skim milk, 0.05% TWEEN 20 and goat anti-rabbit IgG - alkaline phosphatase (AP) conjugate (Sigma) or goat-anti-murine IgG - alkaline phosphatase (AP) conjugate (Sigma) (at 1:1000 dilutions), respectively. After 3 washes in PBS with 0.05% TWEEN 20 as before, blots were soaked in alkaline phosphatase reaction buffer (100 mM Tris-HCl (pH 9.5), 150 mM NaCl, 10 mM MgCl₂) and then developed in 0.05% nitroblue tetrazolium (NBT), 0.05% 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in the same buffer, until signals were clearly visible (approximately 15 minutes).

Please replace the paragraph beginning at page 46, line 6 with the following amended paragraph:

A similar procedure was followed for anti-histidine tag antibody, except that blocking was in 3% bovine serum albumin in TBS (10 mM Tris-HCl, pH 7.5, 150 mM NaCl). Primary and secondary antibodies were diluted in TBS alone, and incubation with primary antibody (1:500 dilution) was for two hours. Washes were performed as follows: blots were first washed twice for 10 min in TBS containing 0.05% ~~Tween~~ TWEEN 20 and 0.2% ~~Triton~~ TRITON X-100, and once for 10 min in TBS.

Applicant : Lee Mizzen *et al.*
Serial No. : 09/001,737
Filed : December 31, 1977
Page : 6

Attorney's Docket No.: 12071-014001

Please replace the paragraph beginning at page 48, line 3 with the following amended paragraph:

Other, similarly prepared filters were incubated with a 1:3000 dilutions of antibodies SPA-807 or SPA-870 for one hour. Blots were rinsed twice with water, washed 3 times (5 min each) with PBS containing 0.05% TWEEN 20 and then incubated for an additional hour in PBS containing 5% skim milk, 0.05% TWEEN 20 and a 1:3000 dilution of goat anti-rabbit IgG - horseradish peroxidase (HRP) conjugate (Sigma). Subsequently, filters were rinsed with water, washed with PBS containing 0.05% TWEEN 20 as before, equilibrated in ECL substrate mixture (Amersham), wrapped in plastic wrap and exposed to X-ray film for between 15 seconds and 20 minutes.

Amendments to the Claims:

The following listing of claims replaces all prior versions and listings of claims in the application.

Listing of Claims:

1. (Cancelled)
2. (Previously Presented) An isolated nucleic acid molecule encoding a *Streptococcus pyogenes* Hsp60.
3. (Previously Presented) An isolated nucleic acid molecule selected from the group consisting of:
 - (a) an isolated nucleic acid molecule comprising the sequence of SEQ ID NO: 5 from nucleotides 15-1649;
 - (b) an isolated nucleic acid molecule comprising the sequence of SEQ ID NO: 7 from nucleotides 15-1652; and
 - (c) an isolated nucleic acid molecule comprising a sequence complementary to the sequence of SEQ ID NO:5 from nucleotides 15-1649 or complementary to the sequence of SEQ ID NO:7 from nucleotides 15-1652.
4. (Currently Amended) An isolated nucleic acid molecule comprising at least 24 nucleotides that hybridizes to SEQ ID NO: 5 from nucleotides 15-1649, ~~SEQ ID NO: 7 from nucleotides 15-1652, or to a complement of SEQ ID NO: 5 from nucleotides 15-1649, or a complement SEQ ID NO: 7 from nucleotides 15-1652~~ when hybridization is carried out at 65°C in 6x SSC, 1x Denhardt's solution, and 0.1% SDS, and washing is carried out at 65°C in 0.2x SSC, 1x Denhardt's solution, and 0.1% SDS.
5. (Currently Amended) ~~The nucleic acid molecule of claim 4, wherein the~~ An isolated nucleic acid molecule ~~comprises~~ comprising a nucleotide sequence that is identical to a segment comprising at least 25% of contiguous nucleotide bases of SEQ ID NO: 5 from nucleotides 15-1649,

SEQ ID NO: 7 from nucleotides 15-1652, a complement of SEQ ID NO: 5 from nucleotides 15-1649, or a complement of SEQ ID NO: 7 from nucleotides 15-1652.

6. (Previously Presented) An isolated nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide comprising a sequence that is at least 95% homologous to SEQ ID NO:6 or SEQ ID NO:8.

7. (Previously Presented) The isolated nucleic acid molecule of claim 3, encoding a polypeptide that is selectively bound by an antibody specific for a *Streptococcus pyogenes* Hsp60.

8. (Currently Amended) An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising a Streptococcal Hsp60 peptide consisting of at least 8 contiguous amino acids selected from amino acid residues 1-544 of SEQ ID NO: 6 ~~and amino acid residues + 545 of SEQ ID NO: 8~~, wherein the Streptococcal Hsp60 peptide binds to a major histocompatibility complex molecule.

9-18. (Cancelled)

19. (Currently Amended) A vector comprising [[an]] the isolated nucleic acid molecule according to of any one of claims 2-8.

20. (Currently Amended) The vector of claim 19, wherein the vector is an expression vector comprising a promoter operatively linked to the isolated nucleic acid molecule molecule.

21. (Currently Amended) The vector ~~according to~~ of claim 20, further comprising a selectable or identifiable marker.

22. (Currently Amended) The vector ~~according~~ claim 20 of claim 20, wherein the promoter is a constitutive or an inducible promoter.

23. (Currently Amended) A host cell containing ~~a vector according to the vector of~~ claim 19.

24. (Currently Amended) The host cell ~~according to claim 24 of claim 23~~, wherein the host cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell and an insect cell.

25-30. (Cancelled)

31. (Currently Amended) A composition comprising [[an]] the isolated nucleic acid molecule of any one of claims 2-8 and a pharmaceutically acceptable carrier or diluent.

32. (Previously Presented) The nucleic acid molecule of claim 3, wherein the nucleic acid molecule comprises nucleotides 15-1649 of SEQ ID NO:5.

33. (Previously Presented) The nucleic acid molecule of claim 3, wherein the nucleic acid molecule comprises nucleotides 15-1652 of SEQ ID NO:7.

34. (Previously Presented) The nucleic acid molecule of claim 6, wherein the polypeptide comprises SEQ ID NO:6.

35. (Previously Presented) The nucleic acid molecule of claim 6, wherein the polypeptide comprises SEQ ID NO:8.

36-37. (Cancelled)

38. (Previously Presented) The nucleic acid molecule of claim 6, wherein the polypeptide comprises an amino acid sequence that is at least 97% homologous to SEQ ID NO:6 or SEQ ID NO:8.

39. (Previously Presented) The nucleic acid molecule of claim 6, wherein the polypeptide comprises an amino acid sequence that is at least 98% homologous to SEQ ID NO:6 or SEQ ID NO:8.

40. (New) The isolated nucleic acid molecule of claim 3, selected from the group consisting of:

- (a) an isolated nucleic acid molecule consisting of the sequence of SEQ ID NO: 5 from nucleotides 15-1649;
- (b) an isolated nucleic acid molecule consisting of the sequence of SEQ ID NO: 7 from nucleotides 15-1652; and
- (c) an isolated nucleic acid molecule consisting of a sequence complementary to the sequence of SEQ ID NO:5 from nucleotides 15-1649 or complementary to the sequence of SEQ ID NO:7 from nucleotides 15-1652.

41. (New) An isolated nucleic acid molecule consisting of 12, 14-18, or 24 nucleotides that hybridizes to SEQ ID NO:7 from nucleotides 15-1652 or to a complement of SEQ ID NO:7 from nucleotides 15-1652 when hybridization is carried out at 65°C in 6x SSC, 1x Denhardt's solution, and 0.1% SDS, and washing is carried out at 65°C in 0.2x SSC, 1x Denhardt's solution, and 0.1% SDS. 42. (New) A vector comprising the isolated nucleic acid molecule of claim 41.

43. (New) The vector of claim 42, wherein the vector is an expression vector comprising a promoter operatively linked to the isolated nucleic acid molecule.

44. (New) A host cell comprising the vector of claim 43.

45. (New) A composition comprising the isolated nucleic acid molecule of claim 41 and a pharmaceutically acceptable carrier or diluent.